Lumio tag (Invitrogen). In some cases, the Lumio tag is reacted with Lumio Green Reagent which is As-derivatized fluorescein, which becomes fluorescent when bound to the Lumio-tagged protein. If the expressed protein does not bind to the sensor molecule, fluorescence may be relatively evenly distributed throughout the droplet. However, if the protein binds to the sensor molecule, fluorescence may be found to concentrate on the bead.

[0185] As another example, a fluorescently labeled ligand which specifically binds the product (and not the substrate) can be used, e.g. an antibody co-encapsulated in the droplet. If the expressed protein does not catalyze transformation of the sensor molecule (substrate) into product, the fluorescently labeled ligand may be relatively evenly distributed throughout the droplet. However, if the expressed protein catalyzes the transformation of the sensor molecule into product, the fluorescently labeled ligand may be found to be concentrated on the bead.

[0186] Fluorescence detection can be performed, in one embodiment, as follows. Using laser illumination and a fluorescence detector, droplets containing a fluorescent bead and those in which the fluorescence is distributed evenly throughout the droplet can be distinguished, and accordingly sorted. It is thus possible to detect and screen against multiple different target molecules by pre-preparing different sensor molecule-bead complexes, where the beads are themselves tagged. A non-limiting example of a suitable bead is a Luminex® bead. Other detection techniques that can be used involve determining binding, e.g., via a change in fluorescence polarization of a fluorescently labeled ligand when bound by the expressed protein, Forster resonance energy transfer (FRET) between the fluorescently labeled expressed protein and a fluorescently labeled, ligand, etc.

[0187] Examples of suitable systems include, but are not limited to, the screening of antibodies produced by hybridomas, human cells (e.g., human blood cells, such as B cells or plasma cells), bacteria or yeast or expressed in vitro (e.g., where the target molecule is an antibody and the signaling entity includes an antigen); or protein-protein interactions.

[0188] The method in this example is high-throughput, enabling drop production and detection on the order of 1 to 10 kHz. Other, higher speeds are also possible. In addition, the method includes a novel system for detecting, e.g., protein-antibody and protein-protein binding, in a fluidic droplet, for instance, via coupled beads or fluorescence intensity detection. Successful matches can be selected and the desired cells can be recovered alive.

[0189] Examples of applications of this example include, but are not limited to, rodent antibodies for research and diagnostics, human therapeutic antibodies, cell lines for antibody production, or technologies for the investigation of protein-protein interactions.

[0190] Another example illustrates the high-throughput expression screening of hybridomas for monoclonal antibody production. Monoclonal antibodies are a valuable biological reagent. They can be used for sensitive detection and quantification of target proteins of interest. Ideally, there would be a monoclonal antibody (or a small collection of monoclonal antibodies) for every protein encoded by a given genome. This would represent a library of roughly 20,000 distinct antibodies. However, the current procedure for the generation of high quality antibodies is tedious, taking about 5-6 months per antibody, at a cost of approximately $5,000/antibody. Typically, a mouse is immunized with a purified protein of interest. Spleens from immunized mice are then dissociated in cell culture to liberate lymphocytes. Lymphocytes are then fused to a myeloma cell line to create immortalized hybridomas, each of which generates a single antibody. The rate-limiting step in the generation of high quality antibodies, in certain cases, is selecting hybridomas that generate antibodies binding to a given protein of interest.

[0191] This example illustrates one method to accomplish this goal in a high-throughput manner. The method described in this example includes an expression screening strategy that makes use of in vitro translated proteins, antibodies from large collections of hybridomas, and microfluidic droplet technology.

[0192] A cDNA library can be subjected to in vitro transcription/translation. New in vitro translation technologies permit translation with incorporation of fluorescence amino acids so that these protein products are fluorescent. For example, in some embodiments, the CCPGCC Lumino tag (Invitrogen) can be used to make in vitro translated proteins fluorescent. Starting with a cDNA library, a large collection of droplets can be created, containing many copies of a single protein, as well as the cDNA, which serves as a barcode for the protein in the droplets. Individual hybridoma cells can be localized in the droplets, where they can secrete antibodies. To allow high-throughput selection of antibodies, hybridomas produced from a mouse can be used that have been immunized with a large number of proteins simultaneously. The secreted antibodies and hybridomas are thus contained within a single "hybridoma droplet." Thus, "hybridoma droplets" can be created containing hybridoma cells as well as secreted antibody, or "IVT droplets" can be created containing cDNA and its fluorescent protein products. Hybridoma and IVT droplets can also be fused together in some cases.

[0193] By beginning with an entire library of hybridoma droplets, as well as an entire cDNA library, an entire library of IVT droplets can be produced. These droplets can be fused and then selected. The droplets can contain a hybridoma, which can now be expanded. The droplets also contain a cDNA barcode, which can be re-sequenced to identify the protein of interest. In this manner, hybridomas can be mapped to the proteins to which their secreted antibodies bind.

[0194] This method involves, as another example, the immunization of a mouse with a complex mixture of proteins. In addition, this method can be run in a high-throughput manner, and can allow for sufficient genome-scale production of antibodies. The method is also based on an expression screening, where a complete cDNA library is translated in vitro and screened for binding to a library of hybridoma antibodies.

EXAMPLE 2

[0195] In this example, microfluidic devices were used to encapsulate, incubate, and manipulate individual cells in picoliter aqueous drops in a carrier fluid at rates of up to several hundred Hz. In this set of embodiments, individual devices were used for each function, thereby increasing the robustness of the system and making it flexible and adaptable to a variety of cell-based assays. The small volumes of the drops enabled the concentrations of secreted molecules to rapidly attain detectable levels. The embodiments described herein showed that single hybridoma cells in 33-pL drops secreted detectable concentrations of antibodies in only 6 hours and remain fully viable.